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Doubling Down on Mutant *RAS* Can MEK or Break Leukemia

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Targeting of the RAS pathway has long been a critical therapeutic challenge in oncology. Burgess et al. examine how the relative expression of mutant and wild-type *KRAS* modulates clonal fitness and sensitivity to MEK inhibitors in a model of *Kras*^{G12D} mutant acute myeloid leukemia and propose its use as a predictive biomarker.

Decades of research on Ras biology have highlighted the central role of this family of small GTPases in human malignancies (reviewed in Karnoub and Weinberg, 2008 and Stephen et al., 2014). Ras proteins transmit signals from cell surface receptors and activate downstream signaling cascades, including the RAF-MEK-ERK and PI3K-AKT pathways, to regulate cell growth, differentiation, survival, motility, and adhesion. Despite tremendous interest, most strategies employed to target RAS have had less efficacy than hoped in clinical trials, due in part to a complex web of alternative pathways and feedback loops. In this issue of *Cell*, Burgess and colleagues demonstrate that murine leukemia cells bearing homozygous *Kras*^{G12D} mutations have a clonal advantage relative to cells with an additional wild-type (WT) *Kras* allele, and a striking sensitivity to MEK inhibitors (Burgess et al., 2017).

Mutations that activate RAS signaling, such as mutations in *RAS* genes (*NRAS*, *KRAS*, or *HRAS*) or RAS regulators (*NF1*, *PTPN11*, or *CBL*), are among the most common genetic abnormalities in cancer. More than 30% of patients with acute myeloid leukemia (AML) bear such mutations, with *NRAS* and *PTPN11* mutations occurring more commonly than *KRAS* mutations (Papaemmanuil et al., 2016). While oncogene mutations are traditionally considered to be dominant, with heterozygous lesions driving malignant transformation, some oncogenes have genetic amplification of the mutant allele, deletion of the WT allele, or uniparental disomy resulting in homozygosity for the oncogenic mutation. For example, mutant oncogenes including RAS and EGFR undergo focal amplification (Beroukhim et al., 2010), and JAK2 mutations are frequently associated with uniparental disomy (Wang et al., 2014). Recurrence of such genomic events suggests that they confer a clonal advantage to a cell. In addition, alterations in the ratio of mutant to WT oncogene alleles may influence response to therapy.

Burgess and colleagues tested the sensitivity of *Kras*^{G12D}- driven mouse models of AML to MEK inhibition. Among the four models studied, all generated using retroviral insertional mutagenesis followed by a conditional hematopoietic-specific knock-in of *Kras*^{G12D}, one model, AML101, showed increased sensitivity to the MEK inhibitor PD0325901 (PD901). In search of the causative link for this enhanced therapeutic response and subsequent evolution of PD901 resistance in this model, the authors performed a suite of analyses in samples pre- and post-PD901 treatment. These studies demonstrated duplication of the *Kras*^{G12D} allele and a loss of WT *Kras* allele in the pretreatment AML101 sample. In leukemia that relapsed following treatment with PD901 (AML101-R), the authors identified trisomy 6 with duplication of mutant *Kras* and a single copy of WT *Kras*. FISH analysis revealed the presence of a small pre-existing trisomy 6 subclone in AML101 prior to therapy, which expanded and became dominant at the time of relapse. Short-term in vivo competition studies demonstrated that AML101 had enhanced clonal expansion relative to AML101-R in the absence of drug treatment, while AML101 was particularly sensitive to PD901 (Figure 1). Similarly, overexpression of WT *Kras* in AML101 rendered it more resistant to PD901 and decreased its competitive fitness.

The authors extended their observations in murine leukemia models to *KRAS*-mutated solid tumor cell lines. They demonstrated that higher mutant *KRAS* allele fraction was associated with increased sensitivity to MEK and ERK inhibition in colorectal cancer (CRC) cell lines, but not in lung and pancreatic cancer cell lines. CRISPR/Cas9 editing of a *KRAS* WT allele to the G13D mutant allele in a *KRAS*^{G13D} heterozygous CRC cell line increased MEK inhibitor sensitivity, as well as RAS-GTPase activity and growth. Finally, analysis of 1,168 primary *KRAS*-mutant tumors across 30 different cancer types identified copy number changes involving the *KRAS* locus, including heterozygous loss of WT *KRAS* in the presence or absence of whole genome duplication, copy-neutral loss of heterozygosity, and genomic gain and amplification of *KRAS* mutant allele in more than half of the cases. Burgess et al. therefore propose the use of ratio of mutant to WT KRAS transcripts as a predictive biomarker in future clinical trials of MEK inhibitors.

Several lines of evidence support the importance of the relative expression of mutant and WT KRAS for tumorigenesis and therapeutic responses. It has been long appreciated that amplification of mutant *Ras* accompanied by loss of WT *Ras* is a frequent event in cancer

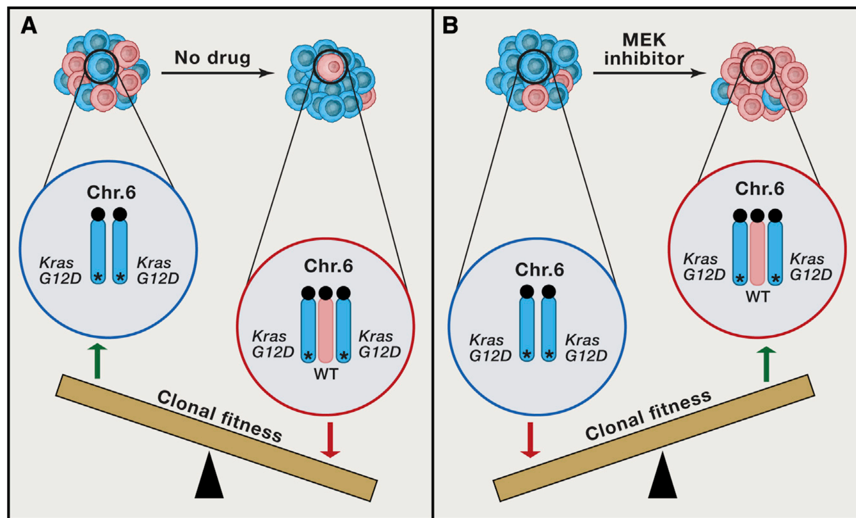

Figure 1. The Effect of Mutant and Wild-Type Kras Expression on Clonal Fitness and MEK Inhibitor Sensitivity

(A) Cells with duplication of the *Kras*^{G12D} allele and a loss of WT *Kras* allele have higher clonal fitness than cells with duplication of the *Kras*^{G12D} allele and a single copy of the WT *Kras* allele in the absence of drug.
(B) Treatment with the MEK inhibitor PD0325901 (PD901) shifts the balance toward increased clonal fitness of the cells with duplication of the *Kras*^{G12D} allele and a single copy of the WT *Kras* allele.

development, suggesting a tumor suppressive effect of the WT Ras protein (Bremner and Balmain, 1990). Bar-Sagi and colleagues demonstrated that decreased expression of WT HRAS or NRAS sensitizes *KRAS*-mutated cancer cell lines to DNA damaging agents (Grabocka et al., 2014). In addition, focal mutant *KRAS* genomic amplifications and elevated KRAS protein expression are associated with sensitivity of cell lines to *KRAS*-targeted shRNAs (Singh et al., 2009). The current study complements these findings and stratifies *KRAS*-mutated cell lines into MEK inhibitor sensitive and resistant using the relative expression of mutant versus WT KRAS. It is possible that the WT *KRAS* allele may mediate MEK inhibitor resistance by reactivation of receptor tyrosine kinases and ERK signaling as has been seen in *BRAF*-mutant tumors treated with RAF inhibitors (Lito et al., 2013). Future studies will be needed to understand the molecular basis of the tissue specificity in Burgess et al.'s observation, and to test whether the same principle applies to different oncogenic *RAS* alleles, some of which preferentially activate different downstream effector pathways.

The findings here indicate that the ratio of mutant and WT *RAS* alleles could be a predictive biomarker for MEK inhibitors. Retrospective analysis of existing datasets from MEK inhibitor trials and carefully designed prospective studies will be critical to address this point. In human AML, the mutant *RAS* allele frequency tends to be low as the mutations are acquired late in disease ontogeny, complicating the determination of the zygosity of *RAS* mutations within an individual cell. Rapid advancement of single-cell technologies, including single-cell DNA- and RNA-sequencing, could be instrumental in this context. Since *RAS* mutations are generally acquired late in leukemogenesis, even complete response of a *RAS*-mutant clone to MEK inhibitor therapy may leave behind highly oncogenic, MEK insensitive ancestral clones. Finally, since oncogenic activation of the RAS pathway may occur through a myriad of different combinations of mutation or copy number alteration of *RAS* genes and regulators, it will be important to understand whether this observation holds true in cases of RAS activation by *NRAS*, *PTPN11*, and *NF1,* which are more frequently mutated and/or lost in AML than *KRAS*.

Studies of genetic predictors of therapeutic response to targeted agents in cancer often focus on associations with particular mutations. The study by Burgess and colleagues illustrates that sensitivity to MEK inhibition is influenced not only by whether a *KRAS* mutation is present, but also by the precise configuration of mutations in *KRAS* alleles. Determination of the ratio of mutant and WT oncogene alleles within a single cancer cell may be critical for prediction of response to targeted cancer therapies.

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